

In Vivo Transfection

In Vivo Transfection Reagents and Delivery Protocols

IN VIVO PEG LIPOSOME NANOPARTICLE LIPID-BASED POLYMER-BASED USES OF DNA
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LIPID-BASED TRANSFECTION

LIPID TRANSFECTION

[Transfection](#) is the introduction of genetic material into a cell. Typically, the genetic material (DNA or RNA) is foreign to the cell and is introduced in order to produce a desired outcome. Most often, this is performed to eukaryotic cells (animal, plant, and fungus cells). Lipids are a type of molecule present in the cell that can create many different organic structures including fats, vitamins and the membrane surrounding organelles. Lipid-based transfection utilizes liposomes to attach to the cell and deposit a desired product.

Liposomes consist of phospholipid bilayers with a spherical shape. Their unique organization enables them to serve as tiny carriers of therapeutic drugs. These small, unilamellar microcontainers are used as carriers for therapeutic drug applications in both pre-clinical and clinical settings for cancer, infections and genetic diseases.

Liposomes deliver their cargo to cells in one of these manners:

- Endocytosis: Transport of particles into a living cell by invagination of the membrane
- Membrane Fusion: Two lipid bilayers join together to form a single entity
- Adsorption: To gather a liposome on the outside surface of a membrane

LIPID-BASED COMPLEXES

Liposomal transfection of cancer cell lines in cell biology research involves use of synthetic molecules that mimic the cellular membrane. These artificial liposomes are common with those found in the cell's membrane, including hydrophilic properties, spherical form and ability to fuse with the membrane. Liposomes are capable to act as a delivery vehicle for nucleic acids, siRNAs (to induce [RNAi](#)), plasmid DNA (to express a gene of interest and/or alter the host cell genome) and multiple other applications. The charge of the liposome determines its attraction to the cell's membrane and fusion interaction for cargo to be absorbed by the cell and released into its cytoplasm.

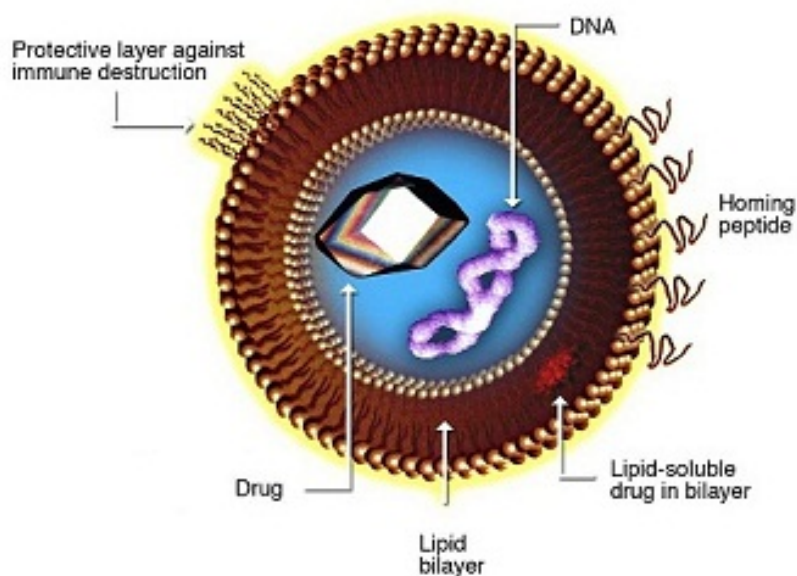
Cationic lipid formulations (see [in vivo lipid-based transfection kits](#)) have been successful in traversing the barriers into the cell, once assembled into an effective cationic lipid/DNA complex. A major benefit of liposome-formulated transfection reagents is that they are biologically inert and biocompatible; thus, they do not yield any unwanted cytotoxic effects.

LIPOSOME STRUCTURE

Basic liposome structure consists of a phospholipid bi-layer with an inner core. Both of these aspects can be customized for specific purposes.

Phospholipid bi-layer:

- Hydrophilic and hydrophobic regions enable protection for inner core payload
- Targeting and tissue specificity moieties can be added to the lipid-soluble layer, such as PEG or antibodies
- Can contain pH sensitive lipids that aid in cytoplasmic release of the active pharmaceutical ingredient (API) upon endocytosis



Source: [Wikipedia](#)

Inner core:

- Aqueous core enables encapsulation of hydrophilic nucleic acids and small molecules
- Can contain any of the following genetic material: RNA, DNA, siRNA, miRNA, aptamers, DNazymes)

LIPID-BASED IN VIVO TRANSFECTION PROTOCOL

LIPID-BASED LIPOSOME DESCRIPTION

Lipid transfection reagent is a [cationic lipid](#) liposome-based formulation optimized for in vivo delivery of small RNA (siRNA, shRNA, microRNA) and plasmid DNA. LIPID in vivo transfection reagent is a proprietary, animal origin-free formulation.

Lipid conjugated complexes are stable in serum for at least 16 hours. There is efficient delivery to the liver, pancreas, kidney, and certain tumor types via systemic administration. siRNA and plasmid DNA can also be injected via subcutaneous tumor injection with minimal toxicity.

Modes of Administration: Tail vein systemic intravenous (i.v.) injection. Direct intratumoral (i.t.) injection. Intraperitoneal (i.p.) injection.

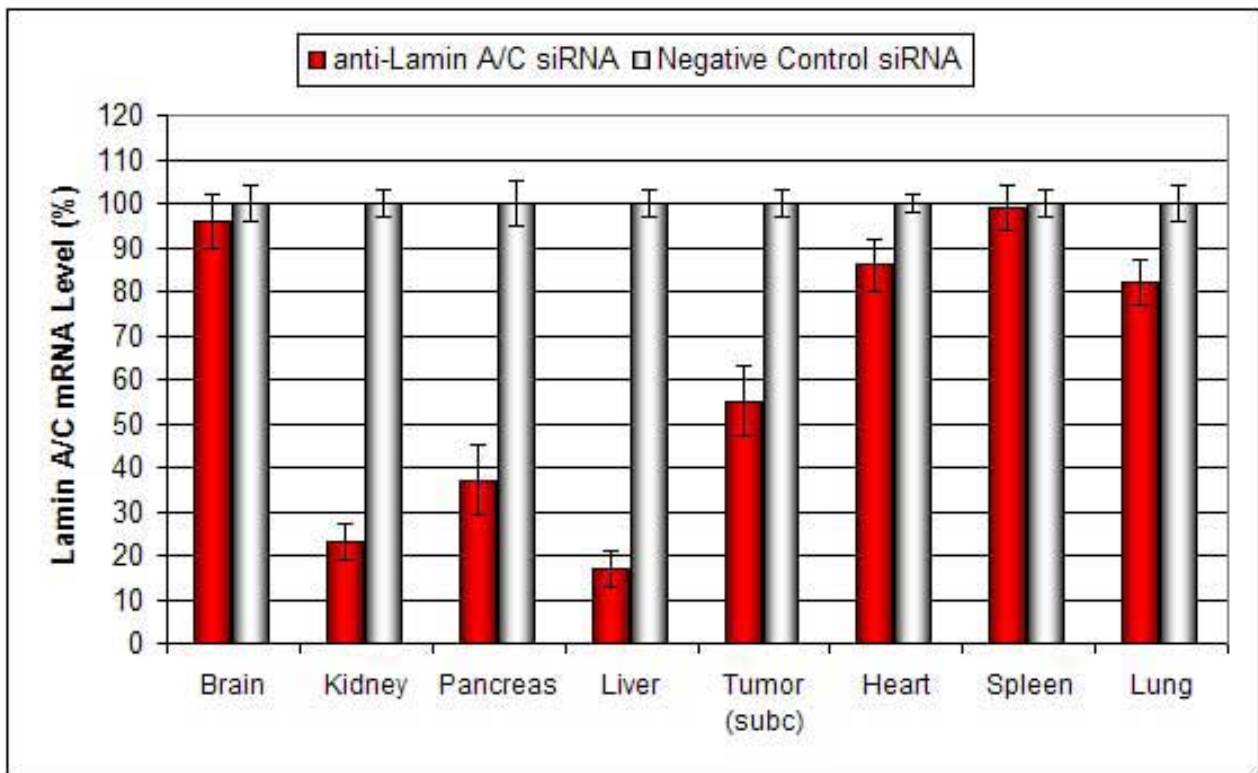


Figure: Systemic administration of Lipid-based *In Vivo* reagent conjugated with a siRNA targeting Lamin A/C (LMNA) mRNA or a non-silencing negative control siRNA. RNA was isolated 48 hours after injection and samples were analyzed by qPCR for LMNA gene expression levels. Data was normalized to a housekeeping gene and expressed as relative to the non-treated control. Data are means \pm SD (n=6).

LIPID-BASED SYSTEMIC ADMINISTRATION

Below is a protocol for the intraperitoneal or intravenous injection of lipid-based complexes for *in vivo* experiments.

1. Dilute 100 μ g of siRNA/miRNA or 60 μ g of plasmid DNA in 100 μ L of nuclease-free water and vortex gently.
2. Add 100 μ L of the diluted nucleic acid to a sterile tube containing 50 μ L Transfection Reagent.
3. Allow the complexes to incubate for 15-20 min at room temperature.
4. Add 10 μ L of the Transfection Enhancer Reagent to the tube and vortex gently to mix.
5. Incubate for 5 min at room temperature.
6. Add required amount of sterile solution of 5% glucose (w/v):

Animal body weight (g)	Final injection volume (ml)
10-14	0.2
15-19	0.3
20-24	0.4
25-29	0.5
30-35	0.6

- Inject animals; optional: delivery efficiency can be increased with a secondary injection.
- Efficacy:** Maximum mRNA target effect is observed 12-36 hours after injection, with maximum effect on protein level achieved 24-48 hours post-injection.

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